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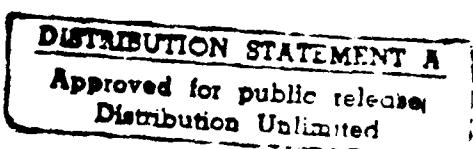
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→ This is a continuation of the annual report  
of the research project "Olfactory Enzymes in the Bovine Olfactory Epithelium".

In the first months of the present research period the following progress was achieved: Hartnett, M., Eglinton, Bonnes, Key, Smith, and Clemons. (3)

### Olfactory-specific proteins gp56 and p52

#### (UDP glucuronyl transferase and cytochrome P450)

In the present period we continued to study the two major specific membrane protein of the bovine olfactory epithelium discovered in our laboratory. The original discovery was made by looking for membrane-associated proteins that will appear in olfactory, but not in the adjacent respiratory mucosa. This approach led to the successful identification of two major proteins: gp56, a glycoprotein of 56kDa, and p52, a non-glycosylated protein of 52kDa. Both were subjected to partial amino acid sequence analysis. The results allowed us to identify the two proteins as members of two families of drug metabolizing enzymes: UDP glucuronyl transferase (UDPGT) and cytochrome P450. On this basis, we proposed a model, whereby such olfactory-enriched enzymes might play a role in: (i) Rapid odorant signal termination and (ii) Protection of the sensory tissue, as well as the immediately adjacent brain, from long term toxicity of odorants. This could be achieved via hydroxylation of odorants by cytochrome P450, followed by the attachment of a charged, hydrophylic glucuronic acid moiety onto the hydroxyl by UDPGT. Such "hydrophyllization" of odorants would allow clearance in analogy to similar processes that underly liver drug detoxification.

In the last few months the following lines of investigation relevant to these enzymes were pursued:

a) *Full length cloning of olfactory UDPGT.*

Continuing the previously reported isolation of a partial length clone of the novel, olfactory-enriched subtype of the enzyme UDPGT, we were able to isolate another clone, #14, which spans the entire coding region and includes 2.1kbases. This clone provides the whole sequence of the olfactory enzyme, and allows us to initiate experiments aimed at functional expression.

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*b) Antibodies against the olfactory drug metabolizing enzymes*

Two rabbit antisera were produced by immunizing with the synthetic peptides shown in table 1, coupled to bovine serum albumin (BSA).

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TABLE 1: Peptides synthesized according to sequence of peptide 19 and peptide 21 of the amino acid sequence analysis . Unassigned amino acids have been replaced by a glycine. A tyrosine residue has been added to the C-terminal of DP21 to enable iodination.

**DP19**    M G Y L P G P Q Q Q A F K E L Q (mix of 4 peptides)

            D                    E

**DP21**    M G K T L T E E K A N R I A S A Y (mix of 4 peptides)

            C                    F

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The antisera were found to specifically recognize the original polypeptides that gave rise to the respective sequences: anti-DP21 reacted specifically with gp56 (UDPGT) and anti-DP19 recognized p52 (cytochrome P450). Both antisera gave positive signals also indirect immunofluorescence localization in frozen bovine and rat nasal tissue sections. They may therefore be used as tools to answer a specific question: what is the cellular and regional local of of the two enzymes within the nasal cavity.

c) *Enzymatic activity of UDPGT in olfactory epithelium.*

(i) *Glucuronation of nitrophenol.*

This substrate is one of the standard ones used to quantitate UDPGT activity. We carried out an in-depth analysis of this activity with olfactory epithelial microsomes. This is the membrane fraction that contains the endoplasmic reticulum, the intracellular membranes that harbor drug metabolizing enzymes in all tissues. Liver microsomes were used for comparison. We used the standard spectrophotometric assay, with a modification that allows to measure activity in micro scale and monitor the results automatically on an ELISA reader.

It was found that olfactory epithelium in the cow and in the rat contained a high nitrophenol glucuronating activity, comparable to that of liver, but at least ten fold higher than in control respiratory nasal epithelium. The  $K_m$  of the olfactory enzyme is however roughly 5 fold higher (weaker) than that of the hepatic enzyme. This may be taken as an indication that the olfactory enzyme is geared to modify compounds other than those that serve as substrates in the liver. We therefore undertook a screening of different substrates in both tissues.

(ii) *Glucuronation of diverse substrates.*

One of the most intriguing questions related to olfactory UDPGT is whether it is capable of modifying odorants. In order to examine this problem, we screened 12 odorants and odorant-like compounds, using a different assay. This second assay was based on monitoring the mobility of radioactively labelled glucuronic acid before and after conjugation to the hydrophobic substrate (aglycone). The mixture of substrate and product is separated on thin layer chromatography (TLC) plates and spot are visualized by autoradiography. This method is excellent for the screening experiment intended, as it is applicable to many different aglycones with practically no modification. This is in contrast with the spectroscopic method described in subsection (i) above, where the absorption properties of the aglycone serve as the basis for monitoring the enzymatic reaction.

The following results were obtained from cow olfactory epithelial microsomes:

- 1) All compounds tested showed activity, that was comparable to the high activity present in liver.
- 2) Well established odorants that contain a hydroxyl group are good substrates. This includes methyl umbelliferone, 1-naphthol, eugenol, linalool, borneol, vanillin, guyacol and citronellol.
- 3) Steroids such as oestrone, testosterone and estradiol are good substrates. A very intriguing finding was that for testosterone, olfactory tissue showed a much higher activity than liver. This could be related to the modification of well known steroid odorants such as androstenone, which is chemically related to testosterone.

In the near future we plan to extend the enzyme assay results and try to determine the specific substrate spectrum of the olfactory enzyme.